

Claims:

1. A polynucleotide sequence having:
 - a) a polynucleotide sequence coding for an amino acid sequence represented by SEQ ID NO: 1;
 - 5 b) a polynucleotide sequence that hybridizes, under stringent conditions, with a polynucleotide sequence coding for an amino acid sequence represented by SEQ ID NO: 1, the amino acid sequence being an amino acid sequence of a protein capable of preferentially producing (S)-4-bromo-3-hydroxy butanoate by asymmetrically reducing
10 4-bromo-3-oxobutanoate; or
 - c) a polynucleotide sequence represented by SEQ ID NO: 2.
2. A DNA construct comprising a promoter in operative linkage with the polynucleotide sequence as defined in Claim 1.
3. A recombinant vector containing the polynucleotide
15 sequence as defined in Claim 1 or 2.
4. A transformant having
the DNA construct as defined in claim 2, or
the vector as defined in Claim 3.
5. A transformant according to Claim 4, wherein the
20 transformant is a microorganism.
6. A transformant according to Claim 5, wherein the microorganism is *E. coli*.
7. A process for producing a transformant, which comprises the step of introducing the recombinant vector as defined in Claim 3 into a host
25 cell.
8. A transformant having the polynucleotide as defined in claim 1.
9. A recombinant vector containing
A) a polynucleotide construct as defined in Claim 1, and

B) a polynucleotide coding for an enzyme capable of converting oxidized β -nicotinamide-adenine dinucleotide phosphate into a reduced form.

10. A recombinant vector according to Claim 9, wherein the enzyme capable of converting oxidized β -nicotinamide-adenine dinucleotide phosphate into a reduced form is a glucose dehydrogenase.

11. A transformant having the vector according to Claim 9 or 10.

12. A transformant according to Claim 11, wherein the host is a microorganism.

13. A transformant according to Claim 12, wherein the microorganism is *E. coli*.

14. A transformant having

A) the polynucleotide as defined in Claim 1, and

B) a polynucleotide coding for an enzyme capable of converting oxidized β -nicotinamide-adenine dinucleotide phosphate into a reduced form.

15. A protein having:

i) an amino acid sequence of SEQ ID NO: 1;

ii) an amino acid sequence encoded by a polynucleotide sequence that hybridizes under stringent conditions with a polynucleotide sequence of SEQ ID NO: 2 coding for an amino acid sequence of a protein capable of preferentially producing (S)-4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate; or

iii) an amino acid sequence of SEQ ID NO: 1, wherein one or more amino acids are deleted, replaced or added, said amino acid sequence being an amino acid sequence of a protein capable of preferentially producing (S)-4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate.

16. A process for producing (S)-4-halo-3-hydroxybutanoate,

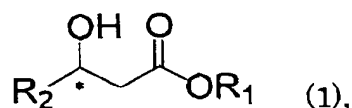
which comprises reacting 4-halo-3-oxobutanoic acid ester with the protein as defined in claim 15, a transformant, which produces said protein or a treated product thereof.

17. A process according to Claim 16, which comprises allowing the coexistence of an enzyme capable of converting the oxidized β -nicotinamide-adenine dinucleotide phosphate into a reduced form.

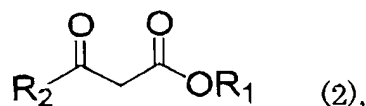
18. A process according to Claim 17, wherein the enzyme capable of converting an oxidized β -nicotinamide-adenine dinucleotide phosphate into a reduced form is a glucose dehydrogenase.

19. A process according to claim 17, wherein the 4-halo-3-oxobutanoic acid ester is contacted with the transformant as defined in any one of Claims 11 to 14 or a treated product thereof.

20. A process according to claim 16, 17, 18 or 19, wherein the 4-halo-3-oxobutanoic acid ester is represented by a formula (1):

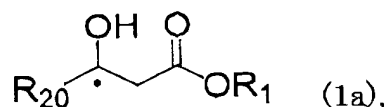


wherein R_1 represents an alkyl group, and R_2 represents a methyl group which is substituted with a halogen atom, which process comprises reacting 4-halo-3-oxobutanoic acid ester of formula (2):

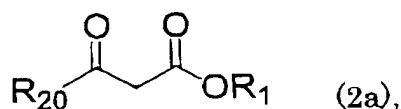


wherein R_1 and R_2 represent the same as defined above.

21. A process for producing an optically active 3-hydroxybutanoic acid ester of formula (1a):



wherein R_1 represents an alkyl group, and R_{20} represents a methyl group which may be substituted with a halogen atom, which process comprises reacting 3-oxobutanoic acid ester of formula (2a):

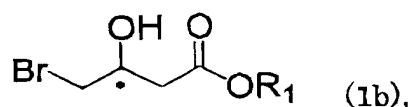


wherein R_1 and R_{20} represent the same as defined above, with a whole cells of a microorganism or a treated product thereof, which microorganism belongs to *Penicillium citrinum*, *Cryptococcus humicolus*, or *Bacillus alvei* and is capable of asymmetrically reducing the oxo group at 3-position of the compound of formula (2a) to corresponding 3-hydroxy group.

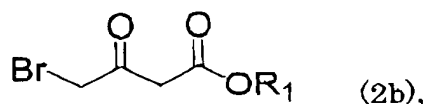
22. A process according to claim 21, wherein R_2 represents a halomethyl group.

23. A process according to claim 21 or 22, wherein the microorganism is a strain selected from the group of *Penicillium citrinum*(IFO4631), *Cryptococcus humicolus*(IFO1527), and *Bacillus alvei*(IFO3343t).

24. A process for producing an optically active 4-bromo-3-hydroxybutanoate of formula (1b):



wherein R_1 represents a (C2-C8)alkyl group, which process comprises reacting 4-bromo-3-oxobutanoate of formula (2b):



wherein R_1 represents the same as defined above, with an enzyme having:

iv) an amino acid sequence of SEQ ID NO: 34;

v) an amino acid sequence encoded by a polynucleotide sequence that hybridizes, under stringent conditions, with a polynucleotide sequence of SEQ ID NO: 34, wherein said amino acid sequence is an amino acid
5 sequence of a protein capable of preferentially producing optically active 4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate; and

vi) an amino acid sequence of SEQ ID NO: 3, wherein one or more amino acids are deleted, replaced or added, said amino acid sequence being
10 an amino acid sequence of a protein capable of preferentially producing optically active 4-bromo-3-hydroxybutanoate by asymmetrically reducing 4-bromo-3-oxobutanoate.

25. A process for producing 4-cyano-3-hydroxybutanoic acid, which comprises reacting 4-bromo-3-hydroxybutanoic acid ester with a metal
15 cyanide in the presence of an alkaline earth metal hydroxide and an alkaline earth metal halogenide.

26. A process according to claim 25, which further comprises the step of reacting the 4-cyano-3-hydroxybutanoic acid with dialkyl sulfate to produce 4-cyano-3-hydroxybutanoic acid alkyl ester.

20 27. A process according to claim 25 or 26, wherein the alkaline earth metal hydroxide is calcium hydroxide, and the alkaline earth metal halogenide is calcium chloride.

28. A process according to claim 25, wherein the 4-bromo-3-hydroxybutanoic acid ester is (C1-C8)alkyl

25 4-bromo-3-hydroxybutanoate, and the dialkyl sulfate is dimethyl or diethyl sulfate.

29. A process according to claim 25 or 26, wherein the 4-bromo-3-hydroxybutanoic acid and 4-cyano-3-hydroxybutanoic acid are optically active compounds.

30. A process according to claim 25 or 26, wherein the 4-bromo-3-hydroxybutanoic acid is (S)-4-bromo-3-hydroxybutanoic acid ester and 4-cyano-3-hydroxybutanoic acid is (R)-4-cyano-3-hydroxybutanoic acid.

5 31. A process for producing (R)-4-cyano-3-hydroxybutanoic acid, which comprises

producing (S)-4-bromo-3-hydroxybutanoic acid ester by asymmetrically reducing the 4-bromo-3-oxobutanoic acid ester, and reacting (S)-4-bromo-3-hydroxybutanoic acid ester with a metal cyanide in the presence of an alkaline earth metal hydroxide and an alkaline earth metal halogenide.

10 32. A process according to claim 31, wherein the asymmetrical reduction is conducted by a microorganism or treated product thereof capable of asymmetrically reducing the 4-bromo-3-oxobutanoic acid ester to (S)-4-bromo-3-hydroxybutanoic acid ester.

15 33. A process according to claim 32, wherein the microorganism is a microorganism belonging to *Penicillium citrinum*.

34. A process according to claim 31, 32 or 33, wherein (S)-4-bromo-3-hydroxybutanoic acid ester and 4-bromo-3-oxobutanoic acid ester are (C1-C8)alkyl ester.

20 35. A process according to claim 33, wherein the microorganism is a strain *Penicillium citrinum* (IFO4631).

36. A process according to any one of claim 31 to 35, wherein the alkaline earth metal hydroxide is calcium hydroxide and the alkaline earth halogenide is calcium chloride.

25 37. A process according to claim 31, which further comprises the step of reacting (R)-4-cyano-3-hydroxybutanoic acid with dialkyl sulfate to produce (R)-4-cyano-3-hydroxybutanoic acid alkyl ester.

38. A process according to claim 32, wherein the alkyl group of

the dialkyl sulfate is a methyl or ethyl group.

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